

CLAIMS

What is claimed is:

1. A method for producing soluble and active recombinant protein comprising the steps of:
 - a. Inserting the p26 beta-core domain into a vector.
 - b. Inserting the insoluble protein domain into the vector directly after the p26 domain.
 - c. Inserting said vector into bacterial cells.
 - d. Growing up the bacteria in a culture to an OD of 0.8 to 1.0
 - e. Inducing said culture with IPTG
2. A method for preventing unwanted proteolysis of a recombinant protein comprising the steps of:
 - a. Inserting bovine alpha-crystallin into a vector.
 - b. Inserting the protein of interest into a vector.
 - c. Inserting said vectors into bacterial cells.
 - d. Growing up the bacteria in a culture to an OD of 0.8 to 1.0.
 - e. Inducing said culture with IPTG.
3. A method for purifying native bovine alpha-crystallin protein. comprising the steps of:
 - a. Homogenizing bovine eye lenses in a buffer.
 - b. Binding alpha-crystallin protein to a Q column
 - c. Eluting the alpha-crystallin with high salt
 - d. Separating the protein in 100 mM Glycine pH 2.5 on a Macroprep ()column
4. A method for purifying recombinant alpha-crystallin type HIS-tagged proteins comprising the steps of:

- a. Inserting the alpha-crystallin protein domain into a vector with the hexa-his tag.
 - b. Inserting said vector into bacterial cells and growing up and inducing said cells.
 - c. Lysing said cells and centrifuging out cell debris.
 - d. Purifying alpha-crystallin protein using a Ni-NTA column.

5. A method for protecting a protein from proteolysis during purification, comprising the steps of:
 - a. Coupling purified bovine alpha-crystallin protein to a chromatography resin.
 CNBr-activated Sepharose 4B
 NHS –activated Sepharose 4B
 - b. Rinsing and blocking said resin with BSA.
 - c. Using said resin to purify the protein of choice.

References:

Ejima D, Watanabe M, Sato Y, Date M, Yamada N, Takahara Y.

High yield refolding and purification process for recombinant human interleukin-6 expressed in *Escherichia coli*.

5 Biotechnol Bioeng. 1999 Feb 5;62(3):301-10.

Gupta P, Waheed SM, Bhatnagar R.

Expression and purification of the recombinant protective antigen of *Bacillus anthracis*.
Protein Expr Purif. 1999 Aug;16(3):369-76.

10

Lim HK, Jung KH, Park DH, Chung SI.

Production characteristics of interferon-alpha using an L-arabinose promoter system in a high-cell-density culture.

Appl Microbiol Biotechnol. 2000 Feb;53(2):201-8.

15

MacRae TH. Formation and function of small heat shock/a-crystallin protein oligomers: Established concepts and emerging ideas. Cell Motil Life Sci. In press 2000.

20

Meyer P, Janin J, Baudet-Nessler S. p55-hGRF, a short natural form of the Ras-GDP exchange factor high yield production and characterization.

Eur J Biochem. 1999 Aug;263(3):806-16.

25 Oneda H, Inouye K. Refolding and recovery of recombinant human matrix metalloproteinase 7 (matrilysin) from inclusion bodies expressed by *Escherichia coli*.

J Biochem (Tokyo). 1999 Nov;126(5):905-11.

30 Ortwerth BJ, Olesen PR. Characterization of the elastase inhibitor properties of α -crystallin and the water-insoluble fraction from bovine lens. Exp Eye Res 1992 Jan; 54(1):103-111.

Sharma KK, Olesen PR, Ortwerth BJ. The binding and inhibition of trypsin by alpha-crystallin. 1992, Biochim Biophys Acta, 1130: 267-76.

- 5 Stevens A., Augusteyn R.C. Isolation of α -crystallin subunits by gel filtration. *Current Eye Research* 1987;6(5):739-740.

- Sachdev D, Chirgwin JM. Order of fusion between bacterial and mammalian proteins can determine solubility in *Escherichia coli*. *Biochem Biophys Res Commun*, 1998 Mar 10 27;224(2): 933-937.

Thies MJ, Pirkel F. Chromatographic purification of the C(H)2 domain of the monoclonal antibody MAK33. *J Chromatogr B Biomed Sci Appl*. 2000 Jan 14;737(1-2):63-9.

15